

FRACTIONATION AND PREPARATION OF FATTY ACIDS AND ESTERS FROM NATURAL MIXTURES WITH THE HELP OF UREA COMPLEXES

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Introduction

The preparation of concentrates of unsaturated fatty acids (or their methyl esters) is an important and basic problem in fat chemistry. Vacuum fractionation, low temperature crystallization and cold storage are usually employed for fractionation of fatty acids, but in countries in which there are no such facilities, the processes involving urea complex formation are indispensable. Schlenk and Holman¹ were apparently the first workers to employ urea complexes in the preparation of purified methyl oleate from olive oil. In order to separate the saturates, these investigators used a stepwise addition of urea which was designed to remove successive 10% portions of olive oil methyl esters as urea complexes. Then they took out one bulk portion representing only methyl oleate. Further purification was accomplished by vacuum fractionation. The purpose of the present investigation was to study the possibility of using urea complex formation alone for concentrating fatty acids of different types in the natural oils, mustard, linseed, cottonseed, sesame, and castor. Initially, some preliminary experiments were conducted to obtain information on separability in mixtures of some known composition. These results were then extended to indigenous oils in order to investigate firstly the feasibilities of isolation of important fatty acids from the oils and secondly to fix optimum conditions for formation of urea complexes with reference to maximum yield and purity of fatty acids in question.

Experimental

Materials.—The methyl esters of cottonseed, linseed and castor oils were obtained by methanolysis through sodium methoxide and methyl alcohol. The acids of sesame and mustard oils were prepared by saponification and subsequent hydrolysis. Methyl oleate (I.N. (Iodine Number) 86.2) was obtained by the methods previously described.² Methyl linoleate (I.N., 172.0) and methyl linolenate (I.N., 259.4) were prepared by bromination and debromination methods³ applied to sesame oil and linseed oil acids.

Apparatus and Methods.—The standard mixture (250 g.) of methyl oleate (I.N., 86.2) and linoleate (I.N., 172.0) and linolenate (I.N., 259.4) in the ratio 1 : 1 : 1, was subjected to urea complex

fractionation by using 250 ml. methanol at the start under reflux and 100 g. of urea for each fraction.⁴ The agitation during complex formation was carried out by a strong and heavy-duty mechanical stirrer. For equal amounts (250 g. each) of other methyl esters, the quantity of methyl alcohol remained the same while that of urea was varied as follows: 100 g. in each of first four fractions of cottonseed and linseed and three castor oil esters and 200 g. in the rest except the last fraction, which represented the filtrate. One hundred grams of urea were used for each of the fractions in sesame oil acids and also for first five fractions in mustard oil acids and then 50 g. for each of the remaining fractions. The urea crystals in each fraction were thoroughly washed with ethyl ether (redistilled over KOH flakes). The wash liquid was freed of solvent and the residue was added to the next proper filtrate obtained during urea complex formation. The fatty acid and ester fractions were recovered by addition of water and a few ml. of dilute hydrochloric acid and then dried over anhydrous sodium sulphate. After analysis, the requisite samples were refractionated through the usual urea complex formation.^{4,5} In case of the standard mixture (Table I), the oleate fraction was subjected to urea complex formation once, (with an equal volume of methyl alcohol per weight of oleate and urea three times its weight), the linoleate fraction four times, and the linolenate fraction, six times. For other natural oil ester and acids, the oleate and oleic acid fractions were each fractionated once by the foregoing method, linoleate and linoleic acid twice, linolenate four times, and erucic acid only once. The urea crystals finally obtained from each fraction were refluxed for one hour with methyl alcohol sufficient for covering the crystals, and then cooled. The crystals were washed in all cases with ethyl ether to remove adhering alcohol and fat components. Rough estimations of linoleic and linolenic acids were made by bromination methods.⁶

Results and Discussion

Table I shows the distribution pattern of fatty acid esters of different unsaturation after urea-complex fractionation of the standard mixture (oleate : linoleate : linolenate = 1 : 1 : 1). On refractionation oleate was obtained from fraction I and II (I.N. 89.7 : ca. 96.0% pure), linoleate from IV and V (I.N., 176.4 : ca. 91.0%), and

linolenate from VI and VII (I.N., 249.1 : ca. 83.0%). The experimental data of Table I on the fractionation of a standard mixture established the trends of the procedures of urea fractionation to be used in the unknown natural mixtures (Table 2.).

TABLE I.—FRACTIONATION OF STANDARD MIXTURE BY UREA COMPLEX FORMATION.

Fractions	% of total ester	I.N.	Fractions containing in major quantities of
I	12.0	96.1	Oleate
II	16.0	101.1	
III	15.0	136.7	Unknown intermediate
IV	12.8	164.0	
V	14.6	190.8	Linoleate
VI	15.7	210.4	Linolenate
VII	13.9	236.4	

Methyl esters of three natural oils used initially for urea complex formation (Table 2) gave better fractionation. In the later fractionations, acids of two oils gave good separation, but some degree of esterification of the acids with methyl alcohol necessitated saponification and hydrolysis of the each fraction to regenerate the total acids. In spite of this difficulty, sometimes acids are found advantageous as starting materials. Table 2 shows the general picture of separation of different fat components with respect to yields and degree of unsaturation in the fractions.

Further purification of the samples in Table 2 by refractionations through urea complexes, yielded reasonably pure fatty acids and esters. Table 3 indicates the purity and yields of different fatty acids and esters after this refractionation. Such purified samples from the local resources would serve various purposes in research in our environments. Further work is in progress to study other advantages of urea-complex formation.

Acknowledgement

The authors wish to thank Dr. Qudrat-i-Khuda and Dr. Salimuzzaman Siddiqui for their encouragement during the progress of the work, and Mr. Waziullah for his help in the experiments.

TABLE 2.—IODINE NUMBER (I.N.) AND CORRESPONDING DISTRIBUTION OF THE UNSATURATES IN THE VARIOUS FRACTIONS OF OILS.

Fractions	METHYL ESTER OF						ACIDS OF			
	Cottonseed Oil		Linseed Oil		Castor Oil		Sesame Oil		Mustard Oil	
	I. N.	% of total ester	I. N.	% of total ester	I. N.	% of total ester	I. N.	% of total acids	I. N.	% of total acids
1	18.5	12.0	95.1	9.6	60.7	8.8	29.5	14.0	52.2	12.8
2	49.5	18.1	108.6	15.7	78.6	11.0	61.2	19.0	73.1	13.2
3	127.1	23.9	169.0	18.0	84.5	18.4	96.5	21.0	84.3	15.6
4	153.9	18.0	178.3	16.0	85.8	21.0	116.6	19.0	87.9	16.6
5	166.5	8.6	189.4	11.8	86.2	40.8	146.2	7.7	92.1	17.2
6	161.6	15.2	230.1	28.9			153.4	8.3	136.2	11.6
7	151.6	4.2					150.5	11.0	181.7	6.4
8									175.4	4.8
9									101.9	1.8

TABLE 3.—CHARACTERISTICS OF SAMPLES OF FATTY ACIDS AND ESTERS PREPARED THROUGH UREA COMPLEXES.

Samples (methyl esters)	I. N.	Yields : % of initial total ester (Table 2)	Sources refractionated (Table 2)
Methyl linoleate ..	167.2	11.2	5th and 6th fractions of cottonseed esters.
Methyl linoleate ..	173.2	14.6	3rd and 4th fractions of linseed esters.
Methyl linolenate ..	244.1	8.4	6th fraction of linseed esters.

(Acids)	I. N.	Yields : % of total acids	Sources as above
Oleic acid ..	91.2	15.6	3rd fraction of sesame oil acids.
Linoleic acid ..	174.5	10.8	5th, 6th and 7th fractions of sesame oil acids.
Erucic acid ..	73.1	16.5	2nd and 3rd fractions of mustard oil acids.
Linoleic acid ..	181.7	5.4	7th and 8th fractions of mustard oil acids.

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